Monocyte Functional Studies in Asymptomatic, Human Immunodeficiency Disease Virus (HIV)-Infected Individuals

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Various aspects of monocyte-associated function were evaluated in the peripheral blood mononuclear cells of male homosexuals who were infected with the human immunodeficiency disease virus (HIV). The functional assessments included indomethacin-sensitive regulation of blastogenesis and lymphokine-activated killer (LAK)-cell induction, chemiluminescent responses of mononuclear leukocytes to opsonized zymosan, and the expression of HLA-DR antigen on CD-14-positive monocytes. The results obtained demonstrate that each of these functions is abnormal in asymptomatic individuals who have HIV core antigen (p24) in their circulation. These results suggest that monocyte abnormalities which could contribute to immune dysfunction in HIV-infected patients can be detected early during the course of HIV infection and are associated with the expression of serum HIV antigen.

KEY WORDS: Monocytes; human immunodeficiency disease virus (HIV); lymphokine-activated killer (LAK) cells.

INTRODUCTION

Infection with the human immunodeficiency virus (HIV), the etiologic agent of the acquired immunodeficiency syndrome (AIDS), is characterized by progressive lymphopenia, particularly in T-helper/inducer lymphocytes (CD4 cells), which results in the development of opportunistic infections and/or malignancies (1–4). HIV is tropic for CD4-positive lymphocytes and CD4-positive monocyte/macrophages (5, 6). While many of the consequences of HIV infection of CD4-positive lymphocytes have been elucidated, studies to assess the consequences of HIV infection of monocytes are only beginning.

Following the demonstration of the CD4 antigen on cells of the monocyte/macrophage and Langerhans lineages (7), evidence was obtained that demonstrated HIV infection of U937 (monocytic leukemia) cell lines and of a macrophage cell line (3801). Also Ho et al. (8) demonstrated the recovery of HIV from the monocytes of asymptomatic homosexual males and patients with AIDS-related complex (ARC). Confirmation of HIV infection in various cells of monocyte/macrophage origin have been obtained by other investigators (9–12).

The possibility that HIV-infected monocytes might exhibit aberrant function has also been suggested. This was originally based on observations in AIDS patients in whom there was demonstrated defective intracellular killing of microorganisms (13), reduced expression of HLA-DR antigens (14), and defective chemotaxis of monocytes (15). In contrast, interleukin-1 (IL-1) production and accessory-cell function reportedly are normal in AIDS patients (16).

In our own studies with monocytes from patients with Kaposi’s sarcoma who did not have opportunistic infections (17), defective responses to opsonized zymosan and abnormal monocyte regulatory function for mitogen-induced blastogenesis and interleukin-2 (IL-2) production were observed.

In the present investigation, we have evaluated certain aspects of monocyte function in asymptomatic HIV-infected homosexual males to determine if the abnormalities we have previously observed in
patients with Kaposi's sarcoma are present in patients at a much earlier stage of HIV infection.

MATERIALS AND METHODS

**Patient Population.** Thirty-six asymptomatic male homosexual patients were evaluated. Of this group, 13 were seronegative for HIV infection (homosexual controls) and 23 were seropositive, as determined by enzyme immunoassay (HTLV-III antibody, EIA; Abbott Laboratories, North Chicago, IL). Nine patients with documented AIDS, six with *Pneumocystis carinii* pneumonia (PCP) alone, and three with PCP and Kaposi's sarcoma (KS) were included in the study for comparison. Twenty-five HIV-seronegative heterosexual men of the same age range were recruited as controls from laboratory personnel at Rush-Presbyterian-St. Luke's Medical Center (heterosexual control group). All patients were tested for HIV p24 antigen (HIV-Ag) in serum using a commercially available EIA (HTLV-HI antigen EIA, Abbott Laboratories, North Chicago, IL). Samples were considered positive whenever the sample contained greater than 50 pg/ml p24 Ag, which is the sensitivity limit of the assay. The lymphocyte subset analysis and HIV-Ag status of our patient population is shown in Table I.

**Isolation of Peripheral Blood Mononuclear Cells (PBMC) and Monocyte Phenotypic Analysis.** Venous blood was aseptically drawn into sterile 10-ml tubes containing 0.5 ml of preservative-free heparin (20 U/ml) diluted with an equal volume of Hank's balanced salt solution (HBSS), layered over lymphocyte separation medium (LSM; Bionetics, Kensington, MD), and centrifuged at 1600 rpm for 30 min. After centrifugation, the mononuclear-cell layer was recovered from the interface and washed twice in HBSS before further manipulation.

For analysis of lymphocyte and monocyte surface phenotype, cells were stained as previously described. Briefly, 1 × 10⁶ mononuclear cells were placed in 6 × 50-mm glass tubes and stained with the appropriate conjugated antibody, phycoerythrin (PE)-labeled Leu-M3 (CD14) or fluorescein isothiocyanate (FITC)-labeled HLA-DR, Leu-4 (CD3), Leu-3 (CD4), or Leu-2 (CD8), (Becton Dickinson Corp., Sunnyvale, CA). The cells were incubated for 15 min at 4°C and washed twice with phosphate-buffered saline, pH 7.2, containing 3% fetal calf serum and 0.1% sodium azide (wash buffer). The wash buffer was aspirated and cells were fixed in a 2% paraformaldehyde solution prior to flow cytometric analysis. Fluorescence was evaluated using an EPICS C flow cytometer (Coulter, Inc., Hialeah, FL) equipped with a 5-W argon laser with 488-nm emission and 600-mW excitation. Filters included a 550-nm dichroic, a 525-nm band pass for FITC detection, and a 570-nm long pass for PE elimination by electronic subtraction. A minimum of 10,000 cells per sample was analyzed. The fluorescence intensity and light-scatter parameters were calibrated daily using fluorescent beads (Coulter, Hialeah, FL) and chicken red blood cells. All data were stored on floppy disks, and additional data analysis was performed on the Terak 8600. Lymphocyte and monocyte populations were identified by their forward and 90° light scatter, and electronic gates were established. Ten thousand cells were analyzed for the gated population. The population was analyzed for Leu-4, Leu-3, and Leu-2 expression on lymphocytes and the coexpression of HLA-DR on Leu-M3.